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Proceedings

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Some Remarks upon Venom and Antivenene.

JOSEPH MCFARLAND, M.D.

Our knowledge of venoms and their effects has passed through three distinct stages in its evolution. In the first, attention was directed to the snakes, their venom apparatus, and the chemical investigation of the venom itself. The second was characterized by careful study of the physiologic action of the venom upon the lower animals, and began about the time that experimental physiology had its inception. The third epoch began with the recent discovery of the phenomena of immunity, immunization, and the therapeutic employment of the antitoxic serums.

Each period has a literature peculiar to itself, with some overlapping, and during all three periods there has been a more or less earnest search after antidotes and cures for snakebites.

It is to some facts that have been determined during the third period that I desire to call your attention this evening.

The occurrence of immunity to serpents' venom seems to have been suspected for many years, but the first to determine its occurrence by experiment seems to have been Sewall, of the University of Michigan, who published in the *Journal of Physiology*, 1887,

vol. viii., p. 203, a paper upon venom, in which he records his success in immunizing pigeons to rattlesnake venom.

The paper seems to have attracted very little attention, and the matter was permitted to rest until Phisalix and Bertrand took it up after interest in immunity and immunization had become general. Their paper appears in the *Compte Rendu de l'Acad. des Sciences de Paris*, 1894, vol. cxviii., p. 288.

This paper was followed by another, *Compte Rendu de l'Acad. des Sciences de Paris*, February 5, 1894, t. cxviii., p. 356, and *Compte Rendu de la Société de Biologie de Paris*, February 10, 1894, 10th series, vol. i., p. 111, in which it was further shown that when guinea-pigs were immunized by venom, a protective (presumably antitoxic) substance appeared in their blood.

The subject of immunization and the formation in the immunized animals of antitoxin (*antivenene*) was greatly elaborated at the hands of Calmette, whose papers in the *Annales de l'Inst. Pasteur*, 1894, vol. viii., p. 275, and vol. ix., p. 225, deserve careful reading, and cover the subject in a most complete manner.

Close upon Calmette came Fraser, of Edinburgh, who, on May 15th, 1895, showed before the Medico-Chirurgical Society of Edinburgh a rabbit immunized to cobra venom to the extent of fifty times the minimum fatal dose, and whose blood serum was protective for other rabbits in doses of $\frac{1}{250}$ of a cubic centimeter.

In the following year Fraser published two large contributions upon the subject (*British Medical Journal*, June 15, 1895, vol. i., p. 1309, and August 17th, vol. ii., p. 416). Our knowledge of the subject as the results of these and foregoing researches may be briefly summarized as follows:

Serpents of certain families are provided with a poison apparatus consisting of a venom (modified parotid) gland, and a fang, or perforated tooth, by which the poison is injected.

The poison is a clear, yellowish fluid, with an acrid odor in some cases, and a slightly oily feel. It is highly irritating when applied to the mucous membranes. It dries readily in the form of scales, which fracture in drying, and readily separate from the polished surface of the dish. Scarcely any of the virulence of the venom is lost by drying.

The activity of the venom depends upon the proteid substances which it contains. One of these can be destroyed by heating to about 80° C. for one-half hour, the other resists 100° C. for some minutes. The different venoms contain different proportions of these principles, and it is probable that the variation in physiologic effects observed depend upon these proportions.

The precipitable principle is thought by most writers to be a *globulin*. Upon its presence the local irritative and neutralizing effects of the venom depend. Venoms such as those of the vipers contain relatively much of this globulin. The other principle which is not easily precipitable is probably an *albumose* (Mitchell and Reichert regarded it as a peptone).

Its chief activity is upon the nervous system, and it is the lethal element of the venom.

The physiologic action of the venom has been fully investigated regarding its action upon the blood, the heart, and the respiration. It causes rapid coagulation of the *blood* when thrown into the circulation in large quantities, so that the animal dies at once; the heart and great vessels are found full of black clots. If the dose is smaller, so that the animal lives for some time, then dies, the blood is black and fluid. If very small doses are given the blood loses some of its coagulability.

Upon the *heart* the venom has but a slight depressing action.

Upon the *respiration* the venom acts profoundly, always causing death by paralytic asphyxia, depending upon the action of the venom upon the respiratory center.

Certain animals, particularly the mongoose, possess a certain degree of natural immunity to venom.

Susceptible animals can be immunized to venom by the usual method of progressive administration, and little difficulty is experienced in increasing this immunity to 50–100 fatal doses.

In the blood of the animals in which this high degree of forced immunity exists, *antivenene*, a substance protective for other animals when injected into them, makes its appearance. This protection is manifested when the antivenene is given before, mixed with, or subsequent to a minimal fatal dose of venom.

The antivenene is useful for therapeutic purposes, and its value can be accurately calculated and expressed as units, and the neces-

sary dose of a serum of proper strength need not exceed twenty cubic centimeters.

The antivenene prepared by immunizing animals to cobra venom operates actively upon the venoms of all known venomous serpents, as well as scorpions, spiders, and some insects.

It is antagonistic to the respiratory or nervous poison, slightly so to the irritative globulin contained in the serum.

The original experiments which I desire briefly to record here were made during 1899 and 1900 upon three horses placed at my disposal through the kindness of H. K. Mulford Co.

The venoms used were almost exclusively those of rattlesnakes furnished by the same firm. Some cobra venoms with which I made some experiments was very kindly sent me from France by Prof. Calmette.

I began my research upon the same plan as Calmette had outlined, and gave my horses some preliminary injections of the cobra venom. Upon consideration, however, it occurred to me that in all probability the serum which I would prepare would be called upon to counteract the effects of crotalus venom, and that it should, if possible, afford protection against the irritative globulins of the crotalus venom. It seemed logical to conclude that serums prepared by the injection into animals of cobra venom which contains some of the irritative globulins, or of heated venoms from which this globulin was all precipitated, would, other things being equal, yield an antivenene active against the nervous poison, and potent to save life, but not potent to prevent the destruction of tissue by the globulins. These thoughts led me to modify the method of immunization, so that I abandoned the cobra venom and subsequently employed crotalus venom only. As the local effects of the venom are so extremely severe, I modified the venom in the beginning by heating it so as to precipitate the globulins, then filtering it. After a period of rapid immunization to the heated venom, which, of course, could only be expected to afford immunity to the nervous poison, a second mode of operation was begun. Dilute solutions of unheated venom were now given subcutaneously. Very small doses were administered at first, but it at once became evident that, while some immunity had been established to the nervous poison, no immunity had

been established to the irritative poison, as each animal immediately after the injection of the venom suffered from a hemorrhagic edema, which sometimes assumed dangerous proportions and led to extensive suppurations and tissue exfoliations. In spite of what was thought to be moderate administration of the venom, two of the horses succumbed to the extremely severe local effects. The impossibility of overcoming these local effects suggested to me that a third modification of the treatment might be brought about, and that by intravenous injection of dilute watery solutions of the venom its further dilution in the circulating blood might enable the animal to tolerate it and lose the local effects. This proved to be the case, and a third period succeeded, during which the remaining horse received diluted crotalus poison, in increasing and then varying doses, into the vein.

To sum up, the treatment of the animal consisted of:

1. The injection of heated cobra venom beneath the skin.
2. The injection of heated crotalus venom beneath the skin.
3. The injection of unheated crotalus venom beneath the skin.
4. The injection of unheated crotalus venom into a vein.

The immunity attained by the horse was highest during the second period, during which it received as much as 3 *grams of the dried venom* at a dose. Of the immunity to the irritative globulin which the animal received during the third period less gratifying results can be reported. The largest quantity given at a dose was 1 gram of the dry venom under the skin, and it almost killed the animal by the enormous slough it occasioned. The recovery was followed by no immunity, as 0.25 gram given later caused a similar edema and slough.

The last period was most successful. The intravenous injection of the unheated venom was conducted with great caution, and I was able to ascend from 0.1 gram to 0.75 gram. The horse, however, always showed a marked and extremely interesting physiologic effect from the venom injections, and when they were increased would fall unconscious for some minutes, then gradually regain consciousness and strength again, some hours elapsing before it was apparently well and ready to eat. The horse fell so suddenly, as the effects of the venom were manifested, that we were obliged to sling him to prevent injury. These phe-

nomena probably depended upon the globulin, as the immunity to the nervous poison was marked, and not enough was contained in the administered venom to injure the animal.

Notwithstanding the fact that these intravenous injections numbered ten, and continued from February to October, 1900, the horse acquired *no immunity* worth mentioning to the irritative globulins, as is fully illustrated by the manner of his death. On October 16, 1900, he received 0.5 gram of the dried, unheated venom into the jugular vein. Through an accident a part (presumably about one-fourth) of the injected fluid entered the subcutaneous tissue near the vein. The result was an edematous swelling of the whole anterior portion of the neck and the development of symptoms of suffocation. Tracheotomy was performed, but the local conditions became so bad that it was impossible to save the animal, and it died.

The horse was bled four times and its serum tested for the presence of antivenene. Unfortunately no bleeding was taken at the end of the time during which heated venom only was used. I think it was probably toward the end of that time that the serum would have been strongest in its protection against the nervous poison.

The first bleeding was taken after the first intravenous injection, and the serum was found to afford an uncertain amount of protection against the lethal dose of venom.

The second bleeding was taken after four intravenous injections, and was found to perfectly protect against the least dose of venom that was fatal in about one-half hour upon intravenous injection, the dose of serum necessary to accomplish this being 1 c.c. It was also found that this serum also afforded very nearly the same amount of protection to the cobra venom sent me by Calmette.

The serum of the third bleeding as well as that of the fourth bleeding failed to protect satisfactorily against cobra venom, so that it had probably declined in strength.

The results of the investigation may be summarized as follows:

1. By the subcutaneous and intravenous injection of unheated crotalus venom it is possible to immunize horses to the chief toxic element—the nervous poison.

2. The immunity thus afforded is very slightly protective

against the irritative poison contained in the venom, and animals whose serum contains much antivenene may succumb to its local effects.

3. The development of the immunity is accompanied by the occurrence of large quantities of antivenene in the blood of the immunized animal.

4. The antivenene is fully able to save animals from both crotalus and cobra venoms.

5. The quantity of antivenene in the blood fluctuates according to circumstances, as do other antitoxic serums, and declines as the vital condition of the horse is depressed.

6. The normal endurance of horses to venoms varies considerably, as does their resistance to diphtheria and tetanus toxins, so that only one out of three horses survived treatment for a considerable length of time.

7. The intravenous method of administering the unmodified venom is by far the best, as it is not attended with sloughing of the tissues. Great care must, however, be exercised in order that the venom does not accidentally enter the subcutaneous tissues.

8. As the immunity does not extend to the irritative poison, and as the protection afforded to animals receiving antivenene is against the venoms, not the irritative poison, it is probable that Calmette's method of immunizing animals with modified (heated) venom, which contains none of the irritative substance, is greatly to be preferred, as causing the animals less suffering and not endangering their lives. *April* 25, 1901.

Diphtheria Bacilli in Noma.*

JOSEPH WALSH, M.D.

Led on by the discovery of Freymuth and Petruschky[1] of diphtheria bacilli in two cases of noma, I examined eight cases of the disease that fell under my observation for the same organism. These cases occurred over a period of two years and a half at St.

* The investigations were carried out in the Pepper Clinical Laboratory. It was through the kindness of Dr. Bryan that I obtained the cases.

Vincent's Home. All of these eight cases showed in cultures the true diphtheria bacillus—that is, the true diphtheria bacillus as measured by the criteria of today. For my differentiation of it from others, and the pseudodiphtheria bacillus, I followed Sternberg as to its growth on different media, and Neisser and others in its staining qualities. Each organism was proven also by inoculation into guinea-pigs or (in one case) a rabbit. The amount inoculated was routinely small, and was taken from a growth on Loeffler's blood serum. Only one of the eight cases showed a pure culture of diphtheria organisms, that the second one was in association with diphtheria. The others showed, beside very large bacilli, diplococci, etc., though I paid attention only to the diphtheria bacilli.

The cases were as follows:

CASE I.—(November 2, 1898.) Helen C., aged three and a half years; mother died of Bright's disease; the child had an acute attack of the same disease at the age of two and a quarter years. On June 28, 1898, she developed diphtheria, and was sent from home to the Municipal Hospital. She returned exactly two months later (August 28th) and the throat still looked diphtheritic, though the cultures were negative. October 27th, two months later, she developed measles, and a day or two later began to whoop. She was at this time in the isolation ward with two other children suffering likewise with measles. One of these two developed what appeared, clinically, to be diphtheria. This one was given 1000 units of antitoxin, and Helen and the third one 500 units each as a prophylactic. Cultures from this case to the Board of Health were negative; my cultures of the same case showed a streptococcus. Two days after the injection of the antitoxin (November 2d) a gangrenous patch was noticed on the interior of Helen's cheek. On November 5th, the day after it was first noticed, the patch was cultured, and it was cultured again on the 7th. Both cultures showed true diphtheria bacilli that killed guinea-pigs in twenty-four to thirty-six hours, the organisms being recovered from the blood and organs in both cases. On account of this finding, on November 9th she was injected with 1500 units of antitoxin, though her condition was very low. November 12th she died. The gangrenous patch, which was in the middle of the

left cheek, measured about one inch in diameter, and extended through all the tissues from the interior outward. The upper alveolus on the left side was entirely necrosed, and there was a perforation through to the nose. Smears from the patch showed with Loeffler's methylene-blue faintly staining bacilli in long threads, small bacilli resembling in general appearance diphtheria bacilli, small and large diplococci, and very large bacilli. Smears stained with Gram's showed very large bacilli and small bacilli and diplococci that were difficult at times to differentiate. A section of the diseased tissue bordering on the healthy, taken after death, showed with Loeffler's methylene-blue many large bacilli and numerous chains of small bacilli, the chains being slightly curved; with Gram's only large bacilli and small diplococci. I carried my isolation attempts no further than the diphtheria bacilli.

CASE II.—(February 6, 1899.) John O'N., aged two and a half years. Well nourished. January 31st he developed pharyngeal diphtheria, two days later noma. The noma appeared on the left upper alveolus, destroyed the gums about two teeth (canine and molar), and perforated through to the nose. Cultures from the pharynx on January 31st showed diphtheria bacilli. The noma patch was cultured first on February 6th, when a pure culture of diphtheria bacilli was found. The child gradually improved, and neither from the pharynx nor the noma patch were diphtheria bacilli found after February 15th. By March 8th the child was almost entirely well. In this case the guinea-pig injected died in about eighteen hours, and I failed to recover the organisms, yet there seemed to be no doubt as to the diagnosis. The guinea-pig was small, weighing 216 grams. The general picture found at the autopsy pointed to the diphtheria bacilli. A second culture from this case showed, beside diphtheria bacilli, very large bacilli which I isolated and injected into a guinea-pig without result.

CASE III.—(April 28, 1899.) Michael M., just over measles (epidemic) when the gangrene came on. It appeared associated with an ulcerative stomatitis almost simultaneously on the left cheek and left alveolar process. It gradually spread till all the teeth fell out, almost the entire alveolar processes sloughed off, with perforation of the palate into the nose, and the destruction of the whole left cheek, nose, and a part of the chin. The child lived four

*

weeks, and practically died of starvation. Cultured four times, on April 12th, 16th, 20th, and 28th; each time the diphtheria bacilli were found. Guinea-pigs were injected twice from cultures of the 12th and 28th.

CASE IV.—(April 28, 1899.) Hugh B., aged four and a half years. Just over measles (epidemic). The gangrene began at the margin of the teeth below lower incisors, in connection with ulcerative stomatitis, spread gradually till it implicated five central teeth and the gums in connection therewith. The alveolar process sloughed to its base. Recovery. Cultured on April 28th, and true diphtheria bacilli found.

CASE V.—(June 11, 1899.) Mary M., aged four years. Had measles immediately previously. The gangrene began below left anterior molar, destroyed all the left lower alveolar process from median line back. Recovery. Cultured June 11; true diphtheria bacilli found.

CASE VI.—(October 1, 1899.) James F., aged five years. Had measles during an epidemic two years before. September 14, 1899, developed diphtheria. Recovered from this, though it is not known if all diphtheria bacilli left his mouth. His digestive tract continued to show disturbances in the shape of a chronic diarrhea and an ulcerative stomatitis. On September 28th a gangrenous patch was noticed on the left upper alveolus. This necrosed almost into the nose, causing the loss of four teeth. Cultures were made on October 1st. They showed the true diphtheria bacillus, which inoculated into a rabbit (no guinea-pigs being at hand) killed it in three days. The bacilli were recovered. The child recovered.

CASE VII.—(January 20, 1900.) Sadie F., aged eighteen months. Teething. Not very well nourished, yet had no previous disease. For the last two weeks has had small hemorrhagic ulcers at the margin of the lower teeth. Yesterday the noma developed. During its progress the lower alveolus necrosed from the median line all the way back on the left side. The lower lip perforated just below the left angle of the mouth. Cultured three different times. Diphtheria bacilli found each time. Guinea-pigs injected twice and bacilli recovered. The bacilli reinjected once and recovered. Child died.

CASE VIII.—(May 21, 1900.) Joseph C., aged four years. The gangrene began at the upper part of the back tooth, spread up the alveolar process, attacked the cheek, perforated and spread over the face. When he died almost the whole face was eaten away, both alveolar processes, both cheeks, the entire nose, and the tissues about the left eye, leaving the eyeball bulging. Cultured May 21, 1900, showed true diphtheria bacilli.

Four of these cases began with an ulcerative stomatitis. This is by no means uncommon, as the text-books indicate (Cabot, in *American System of Practical Medicine*, 1898, and *Twentieth Century Practice of Medicine*). A number of cases, probably fifteen in all, of ulcerative stomatitis were cultured in the hope of finding diphtheria bacilli, but in vain.

The following are examples of such cases:

Fredie (April, 1899), aged three years. Had measles two years before, was suffering again from an attack of the same (I cannot vouch for their being both of the same variety, though the nurse assured me they were). Small hemorrhagic ulcers developed at the border of the lower teeth, and eventually a distinct ulceration of the right angle of the mouth. The cheek and mucous membrane about this ulcer became red and inflamed, gradually became hard and indurated, turned slightly purplish, and we thought we had a case of beginning gangrene. This inflammation continued several days, and gradually disappeared without any destruction. The ulcers at the angle of the mouth and those on the teeth were twice cultured without result as to diphtheria bacilli. Another such case was John P. (September 28, 1899), aged two and a half years. He manifested an ulcerative stomatitis that progressed in its destructiveness until several teeth were loosened and one cheek badly swollen, but no diphtheria bacilli were found on culture, nor did the disease advance to noma. The child gradually recovered.

At this time there were in one ward a number of cases of ulcerative stomatitis manifesting itself principally by ulcers on the gums around the teeth. Many of them were cultured. On one day five and another day four. In no case were diphtheria bacilli found, nor did any case subsequently develop noma.

Other cases of noma reported in the literature in which diphtheria bacilli were found are as follows:

Freymuth and Petruschky[1] two. The first a case of noma genitalium associated with diphtheria of the throat, following measles. The diphtheria and noma were both cured by injections of antitoxin. The second was a child of eight years, suffering from typhoid. The gangrene began on the alveolar process. This was also cured by injections of antitoxin. Bishop and Ryan[2] found in one case what was probably a pseudodiphtheria bacillus, but possibly a true diphtheria bacillus of weak virulence.

As to other investigators, omitting those who were led astray by the organisms of putrefaction and probably indifferent microorganisms and those who made no cultural attempts or got only negative results, I will mention four who found organisms that seem to me possibly to have been causative agents.

Schimmelbusch[3] found in one case cocci, staphylococci, streptococci, and small bacilli. The last predominating, he carried them through a series of inoculation experiments without result; Foote[4] found in one case cultured only on agar, cocci, staphylococci, diplococci, and streptococci. Ranke[5] found streptococci, Guizzetti,[8] in one case, streptococci.

On account of the putrefaction going on in the tissues it is natural to expect that the organisms of putrefaction would be found in large numbers and in association with them numerous other saprophytes. Yet it is very probable that these organisms do not set up the process. Whenever we find moist gangrene, and noma is a typical example of it, we find the gangrene occurring secondarily to some other process, this other process being usually necrotic in character. The important agents then in the etiology of noma would not be the putrefactive organisms, but the organisms that set up the primary necrotic process. The organism that stands out pre-eminently in its power of causing necrosis is the diphtheria bacillus.

Moreover, it is a fact acknowledged by all specialists in children's diseases that diphtheria is especially frequent in association with and after measles. We never have an epidemic of measles at St. Vincent's without seeing some cases of it. If noma may be caused by the diphtheria bacillus, is it not natural that it should be seen especially after those cases when diphtheria itself is common? In a collection of 133 cases from the literature by Hilde-

brandt[6] and Perthes[7], where the previous or associated disease was mentioned, noma occurred with or after measles in 53.

The cases in which streptococci were found I would attribute to them. Streptococci are not so frequent or so saprophytic that they should be found in many cases of gangrene without reason. In the further study of Hildebrandt and Perthes' collection of 133 cases we find noma associated with

Measles	53 times.
Typhoid fever	26 "
Chronic diarrhea	21 "
Scrofula	19 "
Smallpox	9 "
Diphtheria and measles	2 "
Diphtheria and typhoid	1 time.
Diphtheria of the genitalia	1 "
Diphtheria and scarlet fever	1 "

In this list measles comes first, typhoid fever second. I have never seen a case following typhoid fever, yet remembering the capability of the typhoid organism for producing local necroses, I think it might be worth while culturing such a case for the typhoid bacillus itself if the diphtheria bacillus or the streptococcus were not manifest.

The two conditions that follow typhoid fever in frequency, namely, chronic diarrhea and scrofula, mean nothing except as regards the malnutrition of the patient. Then comes smallpox, a disease in which, too, local necroses are common.

It may be asked: "If due so frequently to diphtheria bacilli, why should it not be contagious, at least so far as the diphtheria is concerned?" I would answer that in my small experience I could not say it was not contagious.

As noma and diphtheria follow measles, noma and diphtheria are frequently associated at least at St. Vincent's. From June 1, 1900, to April 1, 1901, we had no case of diphtheria and likewise none of noma. Previous to that time the dates at which children were sent to the Municipal Hospital or died of diphtheria mark the dates of my noma cases.

Many attempts to produce noma by the inoculation of small pieces of diseased tissue into lower animals have failed completely, or have resulted only in the production of small abscesses.

Considered from the stand-point of this paper, this would be natural, since the causative microorganisms, even though still present, would be introduced into a healthy animal in company with large numbers of organisms of putrefaction that might easily destroy them or at least lessen their virulence.

CONCLUSIONS. 1. True diphtheria bacilli are found in connection with many noma cases. Since noma is a species of moist gangrene, requiring probably from analogy two different microorganisms, one a saprophyte to produce the putrefaction, another a parasite to produce the primary necrosis, it is possible that in these cases where diphtheria bacilli are found they may be the primary causative agents.

2. When other pathogenic microorganisms capable of producing necroses are found, it is possible that they may be the primary excitants.

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May 9, 1901.

The Histologic Changes in Diphtheria—A Lantern Slide Demonstration.

RICHARD M. PEARCE, M.D.

The lantern slides used in this demonstration were loaned by Dr. W. T. Councilman, under whose direction they were made, during the preparation of the work which is briefly summarized below.[1]

About sixty lantern slides, illustrating the development and structure of the membrane and the changes in the liver, spleen, kidney, lung, lymph nodes, heart, bone-marrow, and alimentary canal were exhibited.

[1] A Study of the Bacteriology and Pathology of Diphtheria. A Study of 220 Fatal Cases. W. T. Councilman, F. B. Mallory, R. M. Pearce. Journal of the Boston Society of Medical Sciences, vol. v., No. 5.

Summary of Results of Histologic Examination.

Membrane. Diphtheria bacilli are found usually in the necrotic tissue and in the exudation; never in the living tissue or in connection with the primary degenerative lesions of the epithelium. It seems probable that the primary lesions are due to a toxic substance produced by bacilli growing in the fluids of the mouth or throat. When necrosis or injury of the epithelium is produced by this toxic substance the tissue is then invaded by the bacilli. Other microorganisms, particularly the pyogenic cocci, are frequently found associated with the diphtheria bacilli. The first step in the production of the false membrane is degeneration or necrosis of the epithelium, often preceded by direct division of the nuclei of the epithelial cells. An inflammatory exudate, rich in fibrin, coming into contact with this epithelium forms a definite fibrin network. Associated with this, especially on epithelial surfaces having several layers of cells, a hyaline membrane is also frequently formed. This hyaline membrane may be formed from the fusion of the protoplasm of the cells undergoing hyaline degeneration, the nuclei of the cells disappearing and leaving spaces which give the membrane a reticular appearance. This hyaline membrane may also be formed by a hyaline degeneration of exudation cells. The most typical membrane is found in the larynx and trachea. The membrane never forms on intact epithelium, but may extend over it. In the connective tissues and blood-vessels beneath the membrane a hyaline fibrinoid degeneration may occur. Degeneration of the mucous glands is pronounced.

Heart. Fatty degeneration of the muscular fibers was found in forty-six of sixty-seven cases examined. It was most marked in the vicinity of the endocardium. In cases of great severity, dying shortly after entering the hospital, this degeneration was the only lesion of the myocardium. Segmentation and fragmentation of the myocardium was not observed. In thirteen cases very extensive degeneration leading to complete destruction of the muscle fibers was found. This consisted of a fatty degeneration with disappearance of the contractile elements. The sarcous elements disappeared, their place being taken by a granular material, with the formation of large, irregular vacuoles, which could be readily dis-

tinguished from those of fatty degeneration by their size and irregularity.

A further form of degeneration consisted of a complete destruction of the fibrillæ, with the formation of large, irregular hyaline masses. This was found only in the later stages of the disease, the average duration being about fifteen days. It was always more marked near the endocardium. Various degenerative changes of the nuclei of the muscle fibers were noted.

Interstitial lesions occurred in two forms. First, in six cases an acute interstitial myocarditis, characterized by a large number of plasma cells between the muscle fibers, was found. Lymphoid cells were occasionally seen, but polymorphonuclear leukocytes were rare. This condition may or may not be accompanied by degeneration of the muscle fibers. The accumulations were sometimes circumscribed and sometimes diffuse. This condition is analogous to the acute interstitial, non-suppurative nephritis of the kidney. The average duration of this condition was ten days. The second form of myocarditis was more chronic in its character and evidently secondary to degeneration. The interstitial tissue was swollen and infiltrated with large cells of an endothelial character, with considerable formation, in some cases, of connective tissue. The average duration of this condition was seventeen days.

Heart thrombi were found in eight cases. There seems to be some relation between thrombi and the acute interstitial changes of the heart muscle. In seven of these eight cases acute interstitial myocarditis was found.

Lungs. Bronchopneumonia was present in 131 cases, of which in 76 it was discrete and in 55 confluent. The process begins as an infection of the atria, and from there extends; it may be limited to a single acini, to lobules, or to groups of lobules. To the condition in which only single acini are involved, which is essentially the primary lesion, the writers have given the name "acinous pneumonia," to distinguish it from the condition in which the entire lobule is involved. There is little lateral extension of the infection through the walls of the alveoli. Inflammation of the terminal bronchi is also present, but not necessarily of the larger bronchi. Small areas of atelectasis and emphysema were frequently found. Acute lobar pneumonia did not occur in any of

these cases. Inflammatory edema was common, but a general edema of the lungs comparable to the circulatory edema of adults did not occur.

The character of the exudation varies greatly; it may be fibrinous, hemorrhagic, serous, or almost entirely cellular. The cells of the exudation are mainly leukocytes and in part cells derived from proliferation of the lining epithelium. Lymphoid and plasma cells are found not only in the exudation but in many cases infiltrating the interstitial tissue. Necrosis leading to abscess was not an uncommon feature. Dilatation of the lymphatics was very common. No definite relation could be demonstrated between the character of the exudate and the infecting microorganisms. Pneumococci, streptococci, and diphtheria bacilli were found in connection with serous, purulent, fibrinous, and hemorrhagic exudates and with necrosis and abscess formation. Diphtheria bacilli were very frequently found, and may be the cause, without the aid of other microorganisms, of bronchopneumonia, necrosis, and abscess formation.

Spleen. The most obvious lesions of the spleen consisted of the formation of foci of epithelioid cells in the lymph nodes. These epithelioid cells are phagocytic, and the nuclear detritus found in the foci comes generally from the lymphoid cells, which are engulfed and destroyed by the phagocytic cells. Large numbers of plasma cells are found throughout the pulp of the spleen. In the veins an accumulation of lymphoid cells was occasionally found beneath the endothelium of the intima.

Alimentary Canal. In the stomach and intestines, aside from an occasional extension of the membrane, the only important change is the hyperplasia of the lymph nodes with a proliferation of the endothelial cells.

Liver. Lesions of the liver in diphtheria are not characteristic, and do not differ from those found in other infectious diseases; they are due to the action of soluble toxic substances and not to the direct action of the diphtheria bacillus. The most common lesions are fatty and granular degeneration of the liver cells with necroses, which are found chiefly in the centers of the lobules. A slight hyaline degeneration of the capillary walls is seen occasionally, as is also a proliferation of the endothelium.

Kidneys. *Degenerative* changes were found in 112 cases, being chiefly cloudy swelling, fatty degeneration, and hyaline degeneration. Hyaline degeneration was found very commonly and was most marked in the proximal convoluted tubules. *Acute interstitial non-suppurative* nephritis was found in 43 cases. The infiltration was made up chiefly of plasma cells with numerous lymphoid cells and a few polymorphonuclear leukocytes, and occasionally phagocytic and endothelial cells. Various cells were observed which seemed to indicate stages of transition between the lymphoid and plasma cells. The accumulations of cells were generally focal, and were most numerous at the base of cortex adjoining the pyramids, just beneath the capsule, and around the glomeruli. Degenerative changes of the tubules generally accompanied this infiltration, but were not constant. In many of these cases large numbers of the infiltrating cells were found in the bloodvessels. They frequently showed mitotic figures, but not so frequently as did those outside the vessels. Omitting three cases, in which death occurred after the forty-second day, the average duration of the cases in which this condition occurred was sixteen days. In the cases in which the lesion was most marked the average age was eleven and a half days. In younger children infiltration was less marked. *Glomerulonephritis* was found in eleven cases, in nine of which the chief lesion was a proliferation of the cells of the glomular tuft and Bowman's capsule, with more or less lobulation of the glomerulus. It was not possible to resolve any of these cases into the distinct types of intracapillary and capsular glomerulitis; the two conditions were generally combined. In all of these cases there was more or less granular material, evidently coagulated serum, in the capsular space. In the tenth case there was extensive necrosis of the glomeruli with hemorrhage into the capsule, and in the eleventh the capsule was filled with hyaline material, in which both fibrin and red blood-corpuscles were contained. The average duration of these cases was greater than that of those with the interstitial form.

Lymph Nodes. Bronchial, cervical, mesenteric, axillary, and inguinal lymph nodes were examined. The most marked lesions were found in the cervical and bronchial lymph nodes. Two types of lesions were observed: first, those which may follow an injury of almost any sort, consisting of congestion, hemorrhage,

diffuse and circumscribed necrosis; second, lesions which belong distinctly to diphtheria, but which may also be found, in a less marked degree, in any other acute infectious disease of children. These lesions consist of a proliferation of the endothelial cells of the lymph nodules and of the endothelial cells lining the sinuses. These cells are phagocytic, engulfing and destroying principally the lymphoid cells, thus giving rise to the nuclear detritus, which is so characteristic a part of the lesion. These lesions are due to the toxic products of the diphtheria bacilli, the bacilli themselves seldom being found in these structures.

Tonsil. Changes in the lymphoid tissue of the tonsil are similar to those of the lymph nodes generally.

Thymus. The principal change found here was degeneration of the lymphoid cells, especially about Hassall's bodies. The degenerated cells were frequently contained in large cells of the endothelial variety. Eosinophile cells were very numerous.

Bone-marrow. Marked hyperplasia, with great increase of cells resembling plasma cells, was the constant lesion. Lymphoid cells were comparatively few in number, and polymorphonuclear leukocytes still less numerous. Eosinophile cells were very abundant in early cases, less frequent in late cases. Apparent transition forms between the cells resembling plasma cells and the eosinophile cells were seen. A few endothelial cells with phagocytic properties were found. The so-called plasma cells were frequently found in the veins. "It is possible that the ordinary marrow cell is the same as the plasma cell."

Central Nervous System and Skeletal Muscles. Examination in a small number of cases showed local and diffuse fatty degeneration.

The pancreas, adrenals, testicles, thyroid gland, salivary glands, and pituitary body showed no changes of importance.

March 14, 1901.

Experimental Dysentery in Dogs, with Exhibitions of Microscopic Specimens.

H. F. HARRIS, M.D.

During a recent investigation I made numerous attempts to induce experimental inflammations in the intestines of dogs by the rectal injection of various bacteria, and fecal matter from indi-

viduals suffering from dysentery. Among the organisms experimented with are the so-called typhoid bacilli, colon bacilli, the ray fungi, anthrax bacilli, the staphylococci and streptococci of suppuration, the mixed bacteria that were grown from the feces of individuals suffering from dysentery, and the so-called bacillus dysenteriæ of Shiga. As a general rule, several dogs have been experimented upon with each of the organisms mentioned. In a number of cases they were quite young, and therefore presumably somewhat more susceptible than older animals. In order to prevent the injected material from being at once expelled, in almost every instance the dogs received morphin hypodermatically an hour or so before the bacteria were introduced into their intestines, and there can be no doubt that in practically all cases the microorganisms were retained in sufficient quantity to furnish a reasonable test of their pathogenic power. Bouillon cultures of the bacteria were in most instances used for the experiments, though in some cases the organisms were removed from the solid media and thoroughly ground up with sterilized water in a mortar before being injected. As a general rule 10 c.c. of the bouillon cultures were administered, but in some cases the amount was greater and in some less. A record of the time, the quantity of bacteria injected, and the size of the dogs experimented upon was carefully kept in every instance, and the subsequent histories of the animals were likewise preserved. A study of these records shows that not in a single instance did a dog develop an inflammatory condition of the intestine following the injection of bacteria, and in most cases they were entirely healthy for weeks, and even months, thereafter. These results are of much interest, particularly as regards the organism supposed to be the cause of the acute dysentery of the East—the bacillus first described by Shiga. The cultures used were obtained from Prof. Simon Flexner, of the University of Pennsylvania, to whom I desire to express my thanks. It seems significant that these organisms entirely failed to produce any effect on four 10-day-old puppies, into the large intestines of which they were injected, nor did any disturbance follow when they were introduced into the large intestines of three cats.

Much more satisfactory were the results obtained by injecting fresh feces of individuals suffering from dysentery into the large

intestines of puppies, for in every case in which this was done a typical dysentery immediately developed that resulted in the death of the animals, except in one instance, where the dog was killed. In two cases there were several liver abscesses. It should, however, be stated that no ill effects followed the injection of material of this kind into the large intestines of four large adult dogs and in three cats that were almost grown. It would thus seem that puppies are particularly susceptible to the disease-producing cause of this variety of dysentery. These are not the first successful experiments of this kind, for Hlava[1] induced dysentery in two dogs out of seventeen injected with dysenteric material, and in four cats out of six that had been subjected to a like treatment. Kartulis[2] succeeded in producing dysentery in three cats out of seven, he having injected the stools from patients suffering from dysentery, and also cultures of the ameba.

Numerous attempts were made to cultivate the ameba, but in no instance were they successful. It was therefore impossible to determine what the result would be should these microorganisms be introduced in this state into the intestines of animals, but the experiments of Kartulis, already referred to, indicate that the pathogenic agency exists in cultures containing amebæ made from the discharges of patients suffering from dysentery, and he even asserts that the disease was produced in a cat as a result of the introduction of these organisms in a pure form into its intestine. There seems, however, some tendency among writers to doubt the correctness of his statements concerning the latter experiment, for the reason that no one else has so far succeeded in obtaining these parasites in pure culture.

Since it was impossible to cultivate the ameba, it occurred to me that the next best thing would be to attempt to cultivate all of the bacteria in the discharges of dysenteric patients, and to determine if these mixed cultures were capable of setting up a dysenteric process. Cultures were accordingly made from the feces of the same individuals whose discharges had been used to successfully produce dysentery, and these were then injected into

[1] Hlava. Centralbl. f. Bak., Bd. i., 1887.

[2] Kartulis. Einiges ueber die Pathogenese der Dysenterieamoeben. Centralbl. f. Bak., ix., 1891.

the intestines of four puppies. There was absolutely no effect produced. It therefore seems unreasonable to conclude that the germ that produced the disease is a bacterium, or, at any rate, it seems fairly certain that it cannot be an organism that develops in the culture media ordinarily employed. As the latter supposition does not appear at all probable, and as the ameba coli was the only other living organism found in the feces that was probably absent from the cultures, it seems logical to suppose that this parasite is the cause of any morbid state that the injection of these discharges may give rise to. As will be seen in the microscopic section, this view is supported by the fact that the amebæ are abundantly present in and around the ulcers that are found in the intestines of dogs suffering with experimental dysentery, and it does not appear unreasonable to say that the proof is now fairly clear that these organisms are in reality the causative agents in chronic dysentery.

Discussion.

Dr. Flexner wished to congratulate Dr. Harris upon his successful experiments. He stated that the subject of dysentery was at the present time attracting much attention, and he thought that indications pointed to the existence of more than one form of the disease. The distinction into acute and chronic dysentery was not based upon etiology. Doubtless chronic dysentery was merely a later form of the acute disease which might have more than one origin. On the other hand, there were undoubted examples of chronic dysentery in which the history of the acute onset was wanting. It was that class of cases especially that had been found to be associated with the presence of amebæ in the dejecta. He stated that it might be taken as established that the so-called amebic dysentery differs from other forms of dysentery in its pathologic anatomy as well as in its etiology. On the other hand, the conviction had been growing that the acute dysentery *per se* was not due to amebæ but to certain bacterial species; that the acute dysentery might, moreover, become chronic in nature when the pathologic changes of the intestine were so different from the acute changes that without the history of the disease, and bacteriologic examination, the two might not be considered as belonging together.

It was to this form of dysentery that Dr. Flexner wished especially to refer. He stated that while it was a disease prevalent in the tropics, it occurred also in temperate climates, and not improbably might be found to occur in the United States. His own studies of such cases of dysentery occurring in Manila eventuated in the separation of a bacillus from numerous cases differing from the usual inhabitants of the intestinal tracts, agglutinizing with the blood serum of affected individuals, and possessing pathogenic properties for laboratory animals. That organism proved to be identical with a bacillus obtained a short time previous from an epidemic of dysentery in Japan by Shiga. A short time later the organism was obtained from a case of dysentery acquired in Porto Rico, which had lasted for many months, and in which the intestinal lesions, as was afterward shown at autopsy, indicated a chronic process. In a recent publication Kruse, of Bonn, has announced the discovery of an organism probably identical, obtained from an outbreak of dysentery in Germany. Dr. Flexner had recently had an opportunity of studying a case of dysentery in a sailor in Philadelphia in whom he had obtained this organism. The wide distribution of the bacillus, its association with dysentery, its absence from the intestines in a state of health and in some other diseases than dysentery, and the positive specific serum reaction, all rendered highly probable the relation of this bacillus with a particular form of dysentery.

A criticism made by Dr. Harris concerning his experiments made with a culture of the bacillus which Dr. Flexner gave him called for a moment's attention. The bacillus was one which, like so many others, lost its virulence upon long continued saprophytic cultivation. The virulence could, however, be restored by successive passage from animal to animal. In several instances feeding of the bacillus to animals in which the gastric contents had been neutralized caused inflammation of the intestinal tract; but a condition simulating extensive diphtheritic deposits of the intestines in human beings had not been produced experimentally. This was not surprising when it was considered that none of the lower animals were subject to dysentery as it is known in human beings. The imperfection of animals for experiments was, therefore, no just criticism of the importance of this organism in the

etiology of dysentery, and only served to recall similar limitations in the case of the germ of typhoid fever, etc. We were, however, somewhat more fortunate in this case, in that, through an accident, one of Dr. Flexner's laboratory assistants aspirated a small quantity of a culture into his mouth, from which a troublesome and non-fatal form of dysentery was promptly developed; and Surgeon Strong, of the army, had been able to produce the disease in a Filippino prisoner condemned to death who voluntarily submitted himself to the experiment by swallowing a portion of the culture. The organism was recovered from the dejecta.

DR. RIESMAN said that but little could be added to the admirable paper of Dr. Harris and the equally admirable discussion of Dr. Flexner. He had been interested in Dr. Harris' experiments with cats, which evidently seemed less favorable for experiment than puppies. He himself had injected a cat with pus from a dysenteric abscess of the liver, but the cat developed only a transitory diarrhea, and made a complete recovery. The abscess fluid was free from amebæ and was also bacterially sterile. The cat had been injected in the hope that if an ameba was present in an encysted or spore form it might find opportunities for growth in the rectum of the animal, since Quincke and Roos had found the cat very well adapted for the production of experimental dysentery. Dr. Harris was to be congratulated on his success in reproducing in animals the features of human dysentery, even to the production of abscesses. There were very few diseases distinctly human in which this had been done.

DR. HARRIS agreed with Dr. Flexner in holding that there were two forms of dysentery. The amebic form seemed to be a disease of cities, while the other—the so-called "bloody flux" of the South—was common in the country, and rarely became chronic. He thought that the amebic form might begin acutely or subacutely. Whether the amebæ were primarily present or were engrafted secondarily he was unable to say. He did not think that the evidence regarding Shiga's bacillus was positive.

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